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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Steinman et al. EXAMINER: Schwadron, Ronald B.

SERIAL NO.: 09/925,284 ART UNIT: 1644

FILED: August 9, 2001

FOR: ENHANCED ANTIGEN DELIVERY AND MODULATION OF THE IMMUNE RESPONSE THEREFROM

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VIRGINIA 22313-1450

SIR:

I, MICHEL NUSSENZWEIG, hereby declare and state that:

1. I am a Howard Hughes Investigator, Sherman Fairchild Professor and Senior Physician at Rockefeller University having received my Ph.D. degree from the Rockefeller University in 1981 and my M.D. degree from New York University in 1982. I received postdoctoral medical and scientific training at Harvard University. My full curriculum vitae is attached hereto as Exhibit A.

2. My principal area of research is in Immunology and among other positions I serve as reviewer in numerous funding agencies of many countries, including the National Institute of Health, March of Dimes, Dana Foundation. I also have served as reviewer for numerous scientific journals, and I am the Editor of the Journal of Experimental Medicine and the Journal of Immunologic Methods.

3. In the course of my activities, I have been listed as inventor on several patent applications, including the one noted above entitled "ENHANCED ANTIGEN DELIVERY AND MODULATION OF THE IMMUNE RESPONSE THEREFROM", having U.S. Serial Number 09/925,284, which is a continuation-in-part of U.S. application Serial Number 09/586,704, filed on June 5, 2000, which is a continuation of U.S. Serial Number 08/381,528, filed on January 31, 1995, now abandoned.

4. I have reviewed the disclosure of the present application, with particular emphasis on the support in the application as filed for the preparation and generation of antibodies against human and mouse DEC-205 proteins.

5. The present application claims a method of enhancing the development of tolerance to a pre-selected antigen for which tolerance is desired in a mammal comprising exposing ex vivo or in vivo dendritic cells from said mammal to a conjugate comprising said pre-selected antigen covalently bound to an anti-human DEC-205 antibody or an anti-murine DEC-205 antibody that binds to human DEC-205 under conditions that promote dendritic cell quiescence. More particularly, the pre-selected antigen is selected from the group consisting of allergens, autoantigens and antigens participating in allograft rejection. The human DEC-205 protein has a carboxy terminal and amino terminal amino acid sequence as disclosed in the parent application, U.S. Serial Number 09/586,704, filed on June 5, 2000, as SEQ ID NOs.: 1 and 2, respectively. Furthermore, as noted in the parent application USSN 09/586,704, the first 19 amino acid residues of the amino terminal human DEC-205 protein (designated as SEQ ID NO: 13 in the parent application) were used to generate antibodies that reacted with human DEC-205. The sequences from the parent application have now been included in the sequence listing for the present application and are designated as SEQ ID NO: 7 for the carboxy terminal, SEQ ID NO: 8 for the amino terminal, and SEQ ID NO: 9 for the first 19 amino acids of the amino terminal used for antibody generation.

6. The subject matter of the present application was based on work performed in my laboratory, whereby the human DEC-205 molecule was cloned and expressed (Guo, M., Gong, S., Maric, S., Misulovin, Z., Pack, M., Mahnke, K., Nussenzweig, M.C. & Steinman, R.; (2000), A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells, *Human Immunology* 61:729-738). Anti-human DEC-205 antibodies were then prepared by immunizing animals with the first 19 amino acid residues from the N terminal fragment of the cloned human DEC-205 protein.

7. To summarize briefly, the cloning of human DEC-205 was done through use of a cDNA fragment of the 3' portion of mouse DEC-205. This was used to screen a human lymphocyte and thymus cDNA library using standard procedures known to those skilled in the art. In particular, the cDNA fragment of mouse DEC-205 was used to screen a human lymphocyte matchmaker cDNA library (EBV-transformed human peripheral blood B lymphocytes) and a human thymus 5'-stretch plus cDNA library in a Ogt10 vector (Clontech Laboratories, Palo Alto, CA, USA). Positive clones were identified by DNA sequencing on

both strands using Sequenase (United State Biochemical, Cleveland, OH, USA), or the dye determinator kit (PE Applied Biosystems, Foster City, CA, USA) and automated sequencing (Applied Biosystems model 371). The human cDNAs were expressed in pEF-BOS modified to carry a 3' human Fc fragment that was in frame with the insert. DEC-205 leader, CR domain, and FnII domains were amplified from plasmids by PCR using 5' MG31 primers and 3' MG35 primers. The 5' -- primer contains a SpeI site, while the 3' – primer contains a Not site and codes for PRR at the junction point of DEC-205 and the Fc tag. The human DEC-205 Fc fusion protein was produced by transiently transfecting 293 cells using calcium phosphate mediated gene transfer. The fusion protein was purified on protein A sepharose and was then used to inject mice. Following several booster injections, the serum was tested for antibodies that reacted with the CR-FnII domain of the human DEC-205 molecule using Western blot procedures. Afterwards, the spleens were harvested from those animals showing a positive reaction and were fused with Sl'2/0 cells. The supernatants were screened by ELISA, dot blot, thymus tissue staining and FACS analysis. Cell clones that secreted anti-human DEC-205 antibodies were further subcloned and expanded.

8. The present application teaches methods for inducing tolerance by conjugating an antigen to a DEC-205 antibody for targeting to the DEC-205 receptor on specific cells, such as dendritic cells, under conditions that promote dendritic cell quiescence. The antibodies that react with the DEC-205 proteins, in particular, the anti-human DEC-205 antibodies, were prepared using the first 19 amino acid residues from the amino terminal end of the cloned human DEC-205 protein, as described in the parent application U.S. Serial Number 09/586,704, and further attested to in this declaration. Thus, it is my belief that the disclosure of the present application provides sufficient written description for a person skilled in the art to prepare such antibodies that react with human DEC-205 protein as presently claimed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the U.S. Code, Section 1001, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Dated: 1/3/05

Michel Nussenzweig, M.D., Ph.D.

EXHIBIT A

CURRICULUM VITAE

Name: Michel C. Nussenzweig

Date of Birth: February 10, 1955

Education:

1975	B.A. - New York University College of Arts and Sciences
1981	Ph.D. - The Rockefeller University
1982	M.D. - New York University School of Medicine

Clinical Training:

1982-1985	Intern & Resident, Internal Medicine Massachusetts General Hospital
1984-1985	Clinical Fellow, Infectious Diseases Massachusetts General Hospital

Postdoctoral Training:

1986-1989	Harvard Medical School, Department of Genetics

Professional Appointments

1990-1996	Assistant & Associate Professor, The Rockefeller University
1990-1999	Assistant & Associate Investigator, Howard Hughes Medical Institute
1996-present	Professor & Senior Physician, The Rockefeller University
1999-present	Investigator, Howard Hughes Medical Institute
2000-present	Sherman Fairchild Professor of Immunology, The Rockefeller Univ.

Honors & Awards

Summa Cum Laude, New York University College of Arts and Sciences - 1975; Phi Beta Kappa, New York University College of Arts and Sciences - 1975; Alpha Omega Alpha, New York University Medical School - 1982; Bertram M. Gresner Memorial Research Award, New York University School of Medicine – 1982; Elected Member American Society of Clinical Investigators - 1997, Solomon A. Berson Award for Basic Science - 2002

Teaching:

Immunology, Course Organizer

Institutional:

Chair, Transgenic Facility Coordinating Committee
Chair, Animal Care and Use Committee

Chair, Hospital Seminar Committee
Member, Immunology Search Committee
Member, Institutional Review Board for Biohazards, Radioisotopes, Toxic Chemicals, and Carcinogens
Member Hospital GCRC Scientific Advisory Committee
Elected Senior Faculty Representative Academic Council
Member, Virology Search Committee

National
Arthritis Foundation Molecular Immunology study section 1993-1996
NIH Immunobiology Study Section Ad Hoc reviewer 1998, and 1999
NIH ALY Study Section Ad Hoc Reviewer, 1999
NIH NIAID Council Ad Hoc 1998
Organizer Keystone Symposium on Dendritic Cells 1998
Organizer Keystone Symposium on B Cells 1999
March of Dimes Review Committee 1999-
External Reviewer LMGD NICHD 2000
Damon Runyon Cancer Research Fund Review Committee 2000-2002
American Association of Immunologists Program Committee 2000-
NIH ALY Study Section Member 2001-
Organizer Keystone Symposium on B Cell Biology 2003

Editorial:

1996-Present	Editor, The Journal of Experimental Medicine
1999-Present	Editor, The Journal of Immunological Methods
2000-Present	Transmitting Editor, International Immunology
2002-Present	Advisory Editor, Nature Reviews Immunology

Consultant:
Abgenix, Fremont, CA
Zycos, Lexington MA

Professional Memberships:
American Association of Immunologists
American Medical Association
The New York Academy of Sciences
Kunkel Society
Harvey Society

Publications:

1. Steinman, R.M., Witmer, M.D., **Nussenzweig, M.C.**, Chen, L.L. & Cohn, Z.A. Dendritic Cells: an important new cell type in the mixed leukocyte reaction. In: Kaplan JG, ed. Proc. of the 13th International Leukocyte Culture conference. *Elsevier/North Holland Publ.* Co. 273. (1979)

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3. **Nussenzweig, M.C.** & Steinman, R.M. Contribution of dendritic cells to stimulation of the syngeneic mixed leukocyte reaction. *J. Exp. Med.* 151:1196-1212. (1980)

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6. **Nussenzweig, M.C.**, Steinman, R.M., Unkeless, J.C., Witmer, M., Gutchinov, B., & Cohn, Z.A. Studies of the cell surface of mouse dendritic cells and other leukocytes. *J. Exp. Med.* 154:168-187. (1981)

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56. Yu, W., Misulovin, Z., Suh, H., Hardy, R.R., Jankovic, M., Yannoutsos, N & **Nussenzweig, M.C.** Coordinate regulation of RAG1 and RAG2 by cell type-specific DNA elements 5' of RAG2. *Science* 285:1080-1084 (1999)

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*Equal contributing senior author

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